RESPONSE TO OFFICE ACTION

SUMMARY

Tawashi does not teach of or suggest:

1. a biocompatible reductant;
2. an acid having a pKa between 1 and 4;
3. a two phase system , one for the nitrite and one for the acid;
4. aqueous gels.

The pending claims are clearly inventive over Tawashi. Tawashi states, for example, that ferrous sulfate or equivalent water soluble reducing agents such as ferrous chloride, cuprous sulfate or cuprous chloride may be used. Applicants note that the present claimed invention does not teach the use of such metallic salt reductants. It is noted that Tawashi indicates that ferrous sulfate is particularly valuable because it also acts as a "stabilizer for NO". (See Tawashi column 3, lines 5-45). Thus, the present claims 1 and 2 are clearly patentable over Tawashi because they mention no ferrous sulfate or the like, but in its place a "biocompatible reductant." Claims 3 and 4 are likewise even more patentable over Tawashi because they involve a composition comprising "ascorbate salt, ascorbic acid, erythrobate or α-tocopherol reductant." Further, Tawashi teaches nothing of an acid having a pKa between about 1 and about 4. Citric acid is notably deficient in this aspect.

Tawashi teaches solely of nitrite granules and reductant granules in topical or tablet formulations (see Tawashi column 10, lines 1-6; and column 8, lines 16-28). Nothing in Tawashi mentions using powdered or dissolved nitrite salt or reductants, nothing of two gel

systems, and also nothing of aqueous gels. More than one common medium for the nitrite and acid granules is not suggested.

Applicants point out that any acid mentioned by Tawashi is believed to maximize the stability of NO. (See Tawashi column3-4). In this section Tawashi has two equations, one with the presence of acid and one without. In both cases a molecule of nitric oxide is liberated for each molecule of sodium nitrite that is involved. In any case, citric acid does not have sufficient acidity. The effective pKa's for citric acid are above 4. There is certainly no teaching in Tawashi to utilize other acids, particularly such acids that fall within the claims of the present invention (pKa of 1-4). It is also reemphasized that Tawashi teaches the use of mixed granules to make any possibly therapeutic preparations.

Applicants' original and present claims are not suggested by, and are distinguished in a number of aspects from, Tawashi. For example, claims 1 and 2 concern a composition comprising two gels, one having a nitrite salt, and another gel with an acid having a pKa between about 1 and about 4. One of these gels also contains a biocompatible reductant. Claim 10 teaches the use of aqueous gels not taught or suggested by Tawashi. Claim 13 teaches that an ascorbate salt, ascorbic acid, erythrobate or α-tocopherol can be used as a reductant. Other claims dependant upon claims 1 and 2 are likewise even more clearly patentable as particularly pointing out additional features of the present invention. Claim 22 is also patentable for the same reasons mentioned above, most particularly for the presence of two gels in the composition.

Applicants also point out that ferrous sulfate is not biocompatible because it is well known to interact with living tissue in a variety of undesirable modes under normal circumstances. It is, for example, well known to be both an astringent and a gastrointestinal

irritant. Ferrous sulfate is also known, as Tawashi indicates, to coordinate NO, not necessarily a desirable property. The reductants of the present invention are clearly biocompatible and have been long used for treatments involving the human body.

In one aspect, the inventors' choice of preferred maleic acid may have been serendipitous. Maleic acid is considerably stronger than citric acid, thus resulting in an acceptable generation of NO. An important single characteristic of maleic acid is of course that it possesses a pKa lower than 4.0. It should be additionally emphasized that there is no teaching in Tawashi, or even suggestion that separate gels of the present claims, respectively containing any acid and nitric oxide source be utilized. Without such a separation of reactants, even the reactant granules shown in Tawashi would not be stable for extended periods, particularly in the presence of water.

A third important differentiation of an important embodiment of the present invention is that it concerns aqueous gels. Such aqueous gels are medically acceptable, simple to prepare and to utilize, as described in the present application, and do not depend upon an exogenous application of moisture to initiate the generation of nitric oxide. They produce NO upon mixing and resultant diffusion of reactants.

As supported by the accompanying 37 C.F.R. § 1.132 Declaration of Dr. Heggers, the therapy and composition of the present application are surprising and demonstrably effective. Applicants agree that not only is the present-claimed invention non-obvious over Tawashi, it represents surprising and unexpected results. The role of nitric oxide in deleterious health conditions has been ambiguous.

For example, the Stewart, et al., article (*Microsurgery*, 1994; see Exhibit A) indicates that an "excessive, prolonged production of NO contributes to tissue damage in septicemia, ischemia/reperfusion injury, and other inflammatory conditions."

The Payen, et al., article (*Clin. Chest Med.*, 1996; see Exhibit B) indicates that the overproduction of NO in septic shock might result in systemic vasodilation with hyporesponsiveness to vasoconstrictive agents.

The Schaffer, et al., article (*Br. J. Surg.*, 1998; see Exhibit C) indicates that nitric oxide is an immunosuppressive factor that may suppress a normal tissue repair.

The Jude, et al., article (*Diabetologia*, 1999, see Exhibit D) indicates that increased nitric oxide synthase activity in diabetic foot ulcers may be responsible for impaired healing.

The Bruch-Gerharz, et al., article (*Arch. Dermatol. Res.*, 1998 see Exhibit E) indicates that NO has a critical role established for a subset of human skin diseases.

The Bauer, et al., article (*Wound Repair Regen.*, 1998; see Exhibit F) indicates that there exists indeterminant parameters between therapy and toxicity of nitric oxide delivery to wounds.

Importantly, the Paulsen, et al., article (*Wound Repair Regen.*, 1998; see Exhibit G) indicates that nitric oxide can initiate either beneficial or deleterious effects. It was further postulated that keratinocytes within a burn wound would express increased levels of inducible nitric oxide synthase following injury. (Thus, leading to increased levels of nitric oxide.)

The above references before and recently after the filing date of the present application, indicate that there is a likely deleterious effect of nitric oxide on various wounds such as burns and the like. At best, the status would be that the role of nitric oxide in wound healing is

B

ambiguous. Only the present application clearly shows an enhancement of wound healing by the administration of nitric oxide through the present novel nitric oxide delivery systems.

It is emphasized to the examiner that the present invention has a demonstrated efficacy in the acceleration of wound healing. This is specifically shown in a number of examples and under various conditions. That such enhancement of wound healing can be quickly obtained by such nitric oxide generation *in situ* was a fact unknown to Tawashi. Tawashi merely speculated that any condition that would benefit from an increase in peripheral circulation would benefit from the application of his nitric oxide — generating preparation. Such generic teachings, particularly in view of single phase granule preparation, are not useful in the field of medicine, absent some exemplary specificity.

The present claimed invention is patentable on a wide variety of counts, many of which are outlined above. Should the examiner have any suggestions or questions concerning this response, a telephone call to the Applicants' undersigned representative is earnestly requested at (405) 235-7702.

Respectfully submitted,

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EXHIBIT A

as a link with other surrounding platelets, the aggregation process is then amplified and becomes irreversible with the secretion of the platelet intragranular substances through the release reaction. These series of reactions have their own mechanisms of control that regulate or modulate platelet activation as the Ca2+/cAMP relationship, the balance between PGI2 and TxA2 and the enzymatic system of kinases and phosphatases. The relationship between platelets and other blood cells such as erythrocytes and leukocytes probably modulate the platelet aggregatory response during a vascular injury promoting mechanisms of thrombogenesis and wound healing. On the other hand, the multiple compounds released by the endothelial cell that inhibit platelet function, specially prostacyclin (PGI2) and recently the endothelial derived relaxing factor (EDRF) or nitric oxide have focused new understanding on the physiologic role of platelets in the control of hemostatic and thrombotic processes.

Publication Types:

- Review
- Review, academic

PMID: 8054381, UI: 94331450

Other Formats: Citation MEDLINE
Links: Related Articles

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Microsurgery 1994;15(10):693-702

Physiological and pathophysiological roles of nitric oxide.

Stewart AG, Phan LH, Grigoriadis G

Microsurgery Research Centre, St. Vincent's Hospital, Fitzroy, Victoria, Australia.

Nitric oxide (NO), identified as the biochemical messenger of endothelial-dependent relaxation, is of obvious chemical simplicity, but the range and complexity of its biological actions are only now emerging. NO is an important determinant of vascular resistance, it reduces thrombogenicity of the vascular endothelium, contributes to non-specific, host-defence mechanisms, and is a neurotransmitter in the peripheral and central nervous systems. In addition to these physiological roles, there is now convincing evidence that excessive, prolonged production of NO contributes to tissue damage in septicemia, ischemia/reperfusion injury, and other inflammatory conditions.

Publication Types:

- Review
- Review, tutorial

PMID: 7533876, UI: 95191374

Other Formats: Citation MEDLINE

EXHIBIT B

U74389G), and burn-LNMA (20 mg/Kg NG-methyl-L-arginine). The percentage of mean arterial pressure, normalized for the initial value at 30 minutes after the burn injury, decreased in all groups over time but was not significantly different in any group. The normalized percentage of flow also decreased over time in all groups with the slope of the linear regression significantly less in the burn-U74 group (-0.32 95% CI, -0.05, -0.15) and the no burn group (-0.37 95% CI, -0.48, -0.26), compared with the burn-control group (-0.66 95% CI, -0.77, -0.56) and the burn-LNMA group (-0.66 95% CI, -0.77, -0.56). The slope of the linear regression for the normalized percentage of systemic vascular resistance was significantly more marked in the burn-control group (2.45 95% CI, 1.35, 3.54) and the burn-LNMA group (1.22 95% CI, 0.89, 1.55) compared with the no burn group (0.16 95% CI, 0.11, 0.44) or the burn-U74 group (0.34 95% CI, 0.06, 0.74). The burn shock resulted in hemodynamic instability as measured with increased systemic vascular resistance, decreased cardiac output, and mean arterial pressure. Use of a lazaroid (U74389G), not a nitric oxide synthetase inhibitor (NG-methyl-L-arginine), altered the clinical course after thermal injury. These data suggest the importance of lipid peroxidation and free radicals as secondary mediators in the evolution of burn shock.

PMID: 8844348, UI: 97001356

Other Formats: Citation | MEDLINE
Links: Related Articles

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Clin Chest Med 1996 Jun;17(2):333-50

Nitric oxide in sepsis.

Payen D, Bernard C, Beloucif S

Department of Anesthesiology and Critical Care Medicine, Lariboisiere University Hospital, Paris, France.

The synthesis of nitric oxide (NO) and its targets are reviewed physiologically during sepsis and wound healing, a self-limiting process in which mechanisms are still identified incompletely. NO also plays an active and direct role during infection, aimed at protecting the host and destroying the microbe. During septic shock, an overproduction of NO has been described experimentally and clinically that might be responsible for the systemic vasodilatation with hyporesponsiveness to exogenous vasoconstrictive agents. The different manipulations of NO pathway during sepsis are described (transcription and post-transcription of iNOS, enzymatic function, substrate availability, NO concentration, and NO effector molecules), although their clinical benefit remains controversial.

Publication Types:

- Review
- Review, tutorial

PMID: 8792070, UI: 96384173

EXHIBIT C

[Thrombin--a regulator of reparative processes in wound healing].

[Article in Russian]

Strukova SM, Dugina TN, Chistov IV, Markvicheva EA, Kuptsova SV, Kolokol'chikova EG, Rumsh LD, Zubov VP, Gluza E

Department of Human and Animal Physiology, Moscow State University, Russia.

Thrombin, binding to receptors of the protease activated receptor (PAR) family, is involved in wound healing by inducing the reparation processes and regulating the activity of mast cells, which secrete mediators of inflammation. Using thrombin receptor agonist peptide (TRAP-6) for the activation of rat mast cells, effect of several receptors, including PAR-1, on mast cells was demonstrated. It was shown that TRAP increases the concentration of Ca2+ in the cytoplasm of mast cells and regulates cell degranulation, while releasing nitrogen oxide. Thrombin encapsulated in poly(N-vinyl caprolactam)-calcium alginate (PVCL-Ca-Alg) hydrogel films promotes wound healing in rats as demonstrated by the acceleration of fibroblast proliferation and neovascularization.

PMID: 9612571, UI: 98275518

Br J Surg 1998 Apr;85(4):444-60

Lymphocyte function in wound healing and following injury.

Schaffer M, Barbul A

Department of Surgery, Eberhard Karls Universitat, Tubingen, Germany.

BACKGROUND: Injury activates a cascade of local and systemic immune responses. METHODS: A literature review was undertaken of lymphocyte function in wound healing and following injury. RESULTS: Lymphocytes are not required for the initiation of wound healing, but an intact cellular immune response is essential for a normal outcome of tissue repair. Injury affects lymphocyte immune mechanisms leading to generalized immunosuppression which, in turn, increases host susceptibility to infection and sepsis. Although the exact origin of post-traumatic immunosuppression remains unknown, stress hormones and immunosuppressive factors, such as inflammatory cytokines, prostaglandin E2 and nitric oxide, affect lymphocyte function adversely. Post-traumatic impairment of T lymphocyte immune function is reflected in decreased lymphocyte numbers, as well as altered T cell phenotype and activity. Antibody-producing B lymphocytes are variably affected by injury, probably secondary to alterations of T lymphocyte function, as a result of their close interaction with helper T cells. Therapeutic modulation of the host immune response may include non-specific and specific interventions to improve overall defence mechanisms. CONCLUSION: Early resuscitation to restore lymphocyte function after injury is important

EXHIBIT D

20 citations found

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Diabetologia 1999 Jun;42(6):748-57

The role of nitric oxide synthase isoforms and arginase in the pathogenesis of diabetic foot ulcers: possible modulatory effects by transforming growth factor beta 1.

Jude EB, Boulton AJ, Ferguson MW, Appleton I

The Department of Medicine/Diabetes, Manchester Royal Infirmary, UK.

[Medline record in process]

AIMS/HYPOTHESIS: L-arginine, an amino acid involved in wound healing, is metabolised by one of two pathways; nitric oxide synthase and arginase. If metabolised by nitric oxide synthase, this can result in tissue destruction, or matrix deposition if metabolised by arginase. The aim therefore was to investigate the role of these enzymes in the pathogenesis of diabetic foot ulcers. METHODS: The activity, proteins by Western blot analysis and cellular distribution (using immunocytochemistry) of these enzymes were measured in diabetic foot ulcers, diabetic skin and normal skin. RESULTS: Total and inducible nitric oxide synthase ($p < 0.001$) and endothelial nitric oxide synthase were increased in diabetic ulcers compared with diabetic and normal skin and were associated with increased plasma nitrite concentrations in diabetic ulcers ($p < 0.05$). Inducible nitric oxide synthase was the major isoform, with the macrophage being the predominant cellular source. Similarly arginase activity was increased ($p < 0.01$) in diabetic ulcers. The protein levels corroborated with the activity data, with the fibroblast being the major cellular source. The spatial and cellular distribution of the two enzyme systems was distinct. Transforming growth factor-beta1 was decreased in diabetic ulcers in comparison with diabetic skin and normal skin. CONCLUSION/INTERPRETATION: Increased nitric oxide synthase activity in diabetic foot ulcers may be responsible for the impaired healing in this disease. Furthermore, the increased activity of arginase could account for the characteristic callus formation around these ulcers. In addition, the lower concentrations of transforming growth factor-beta1 in diabetic ulcers may explain the raised concentrations of nitric oxide in this condition.

PMID: 10382596, UI: 99309969

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EXHIBIT E

Vitamin B12a (hydroxocobalamin), a natural product, offers an ideal environment to serve as a donor of nitric oxide.

Publication Types:

- Review
- Review, tutorial

PMID: 9881839, UI: 99096166

Other Formats: Citation MEDLINE
Links: Related Articles

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Arch Dermatol Res 1998 Dec;290(12):643-51

Nitric oxide and its implications in skin homeostasis and disease - a review.

Bruch-Gerharz D, Ruzicka T, Kolb-Bachofen V

Department of Dermatology, Heinrich-Heine-University, Duesseldorf, Germany.

The recent identification of the nitric oxide synthase (NOS) pathway in various cell types in the skin has provided important insight into the molecular mechanisms underlying regulatory and homeostatic functions of the skin. Many studies also point to perturbations or defects in the signaling cascade of nitric oxide (NO) and reactive nitrogen intermediates as key players in skin disease pathogenesis. A critical role for NO is now established for a subset of human skin diseases, and new mechanism-based therapies may be available in the near future. This remarkable progress and the implications it may have for common forms of skin disease are reviewed here.

Publication Types:

- Review
- Review, tutorial

PMID: 9879832, UI: 99094441

Other Formats: Citation MEDLINE
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J Clin Gastroenterol 1998;27 Suppl 1:S80-6

EXHIBIT F

Other Formats: Citation MEDLINE
Links: Related Articles

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Wound Repair Regen 1998 Nov-Dec;6(6):569-77

Evaluation of linear polyethyleneimine/nitric oxide adduct on wound repair: therapy versus toxicity.

Bauer JA, Rao W, Smith DJ

Department of Chemistry, University of Akron, Ohio 44325-3601, USA.

A full-thickness wound model was used to evaluate the effects of a topically applied polyethyleneimine-based nitric oxide donor on wound repair in aged rats. Polymer applications were applied over a 10-day period on days 0, 2, 4, 6, and 8 comparing treatment (linear polyethyleneimine-nitric oxide) and control groups (linear polyethyleneimine). Urinary nitrate excretion was quantified as a measure of nitric oxide released. The nitric oxide released from the linear polyethyleneimine-nitric oxide group was significant compared with controls ($p </= 0.001$), with a maximal nitrate level of 40 micromol on day 1 and an average sustained delivery of 34 micromol/day for the remainder of the study. Wound closure was examined using a computer-based video-imaging analysis system. The wounds of both the linear polyethyleneimine- nitric oxide treatment and linear polyethyleneimine control groups exhibited minimal wound closure; however, the wound closure of the treatment group was significant as compared with the control group ($p </= 0.05$). A phosphate- buffered saline solution-wounded control was performed that showed cleaner and faster healing wounds, similar to normal healing, than either of the polymer application groups. The histological data showed very little wound healing, on a cellular level, implicating the linear polyethyleneimine-nitric oxide as well as the carrier compound as contributing to the adverse tissue reactions that occurred in the wound bed. Thus, we report the toxic effects of a polyethyleneimine-based compound, as well as the toxic effects of sustained delivery of excess levels of nitric oxide on the wound- repair process. Our findings suggest that there exists indeterminate parameters between therapy and toxicity of nitric oxide delivery to wounds.

PMID: 9893176, UI: 99113027

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Am J Physiol 1999 Jan;276(1 Pt 1):G238-48

Cigarette smoking delays ulcer healing: role of constitutive nitric oxide synthase in rat stomach.

EXHIBIT G

Shekhter AB, Kabisov RK, Pekshev AV, Kozlov NP, Perov IuL

Publication Types:

- Clinical trial

PMID: 9777240, UI: 98450403

Wound Repair Regen 1998 Mar-Apr;6(2):142-8

Expression of inducible nitric oxide synthase in human burn wounds.

Paulsen SM, Wurster SH, Nanney LB

Departments of Plastic Surgery and Cell Biology, Vanderbilt University Medical Center, Nashville, TN, USA.

Nitric oxide is produced by various cell types and can initiate either beneficial or deleterious effects. Because cultured human keratinocytes express an inducible isoform of nitric oxide synthase, it was postulated that keratinocytes within a burn wound would express increased levels of inducible nitric oxide synthase following the injury. Immunohistochemical staining identified the sites of cellular expression and temporal sequence of inducible nitric oxide synthase protein within partial- and full-thickness burns excised from 29 patients. While migrating keratinocytes at the immediate edge of the wounds showed decreased or undetectable levels of inducible nitric oxide synthase, the immediately adjacent proliferative population and upwardly growing keratinocytes from surviving hair follicles showed increasingly greater cytoplasmic staining for inducible nitric oxide synthase at 4-21 days after injury. Noninjured skin showed minimal inducible nitric oxide synthase staining. Within the wound, detectable inducible nitric oxide synthase protein appeared to decrease as keratinocytes assumed a differentiated phenotype in the outer newly resurfaced epidermis, in inner root sheath layers of hair follicles, or in epithelium of eccrine sweat ducts. Within granulation tissue, immunoreactive inducible nitric oxide synthase was detected in capillary endothelium and in arterial smooth muscle layer. Focal increases in inducible nitric oxide synthase expression were noted in association with inflammatory infiltrates. In conclusion, the cellular and temporal distributions of immunoreactive inducible nitric oxide synthase suggest that nitric oxide may play a role in the regulation of wound repair processes beyond the acute burn injury.

Publication Types:

- Clinical trial
- Controlled clinical trial